VOL. XVI, No. 5 SEPTEMBER-OCTOBER, 1955

UNIVERSITY OF MICHIGAN
JAN 5 - 1956
MEDICAL LIBRARY

610.5
B73

gastro
13, 14, 15

BORDEN'S REVIEW

of

NUTRITION RESEARCH

DIETARY INTERRELATIONSHIPS AND IMBALANCE IN NUTRITION

BORDEN'S REVIEW
of
NUTRITION RESEARCH

Reviews and summaries of research information concerning nutrition and foods. Published bi-monthly, by THE BORDEN COMPANY; officers of the company: Theodore G. Montague, president; E. L. Noetzel, treasurer; D. T. Orton, secretary. Editorial office: 350 Madison Avenue, New York 17, N. Y.

DIETARY INTERRELATIONSHIPS AND IMBALANCE IN NUTRITION

By C. A. ELVEHJEM, Ph.D.* and W. A. KREHL, Ph.D.**

INTRODUCTION

The unceasing efforts of nutritionists have been directed toward employing properly balanced diets to maintain man's health and his natural resistance or defense mechanisms at the peak of operational efficiency. There has also developed the concept that by proper dietary management, injuries to the body induced through nutritional sins of omission or commission may in part be repaired. Utopia for the nutritionist might be visualized as the attainment of optimum nutrition for all. This may be described as that balance of foodstuffs which provides all the dietary nutrients in respect to kind and amount and in proper state of combination (or balance) so that the organism may meet the varied exogenous and endogenous stresses of life, whether in health or disease, with a minimal demand or strain on the body's natural homeostatic mechanisms.

It is of course recognized that such a balanced diet is quite beyond the reach of probably two-thirds of the world's population, whose main concern is still to get just enough calories. Certainly the political efforts to achieve and maintain a free world must take cognizance of this fact. However, in areas of the world where the food supply is both abundant and varied the nutritionist has a responsibility to educate the public concerning food selection, so as to provide an adequate and balanced diet without overnutrition, which is undoubtedly as menacing a form of malnutrition as is undernutrition or the deficiency diseases.

Nutritional Standards

The most practical guides that are available in this country for judging nutritional adequacy are the tables of recommended dietary allowances prepared by the Food and Nutrition Board of the National Research Council (1) and the minimum daily requirements of specific nutrients of the U. S. Food and Drug Administration. The recommended dietary allowances have been based on experiments with laboratory animals, clinical observations, and considered judgment based on evaluation of extensive nutritional literature. These figures provide a substantial safety factor well above the mini-

*Professor of Biochemistry, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin.
**Associate Professor of Nutrition, Yale Nutrition Laboratory, Yale University, New Haven, Connecticut.

mum needs for most people. Certainly these tables have been of great value in the modern development of good dietary practices.

The report of Pollack and Halpern (2) on the nutritional needs of sick, wounded and convalescent patients emphasizes that many such patients require and respond favorably to levels of nutrients considerably in excess of the recommended levels of dietary allowances. This concept of therapeutic nutrition recognizes the flexibility of nutritional needs based upon the stresses to which the individual is exposed.

R. J. Williams has focused attention (3) on the considerable biological variability and the highly individual characteristics of man in considering nutritional needs. This concept points up the limitations of applying average yardsticks of nutritional adequacy when considering the individual. Most of our dietary surveys and nutritional studies with human subjects have been, justifiably, on a mass or large group basis. Perhaps it is time to consider nutritional needs on the basis of the individual, just as the physician must consider each patient as a separate entity.

From what has been said above it is quite conceivable that the plane of optimum nutrition represented by a balanced diet may or may not fall within the limits of the recommended dietary allowances.

Homeostasis and Stress

Fortunately, man is able to compensate and adapt, within limits, to unfavorable environmental and dietary circumstances. This is possible in part because of the ability of the organism to call into play the mechanisms of homeostasis which maintain what Claude Bernard described as the "constancy of the milieu interieur."

Numerous and complex alterations of metabolic patterns enable man to function during stress. In starvation, for example, the fat stores of the body are mobilized to supply caloric needs and the all-important blood glucose level is maintained by the process of gluconeogenesis. It is obvious that these are emergency measures, which, if continued, will lead to pathology.

It is visualized, therefore, that the availability of a balanced diet provides for the maintenance of metabolic normalcy and places a minimal demand on those mechanisms which are kept in reserve, ready to meet the emergency of stress.

The Balanced Diet and Toxic Factors in Nutrition

In considering the balanced diet, calories, carbohydrate, fat, ash, vitamin and protein content must be considered individually and with respect to each other.

As in a previous report of this subject (4) frank nutritional deficiencies and toxic factors in the diet will not be discussed at length as factors in nutritional imbalance. Certainly substances in food, whether naturally present or introduced as the result of processing, such as fluorine, selenium, arsenic, copper and lead, may cause serious damage if present in foods at too high a level. Fortunately, because of the diligence exercised by both the food industry and the U. S. Food and Drug Administration, the problem of toxic materials in our food supply is minimal.

The problem of chemical additives is too vexing and complicated to be discussed here, but must be kept in mind when considering dietary imbalance.

Mineral Balance

The ratio of calcium to phosphorus in the diet is undoubtedly of great concern in regard to the utilization of each of these elements. Considerable amounts of these minerals may be lost in the feces and hence be unavailable if there is appreciable distortion of the normal calcium-phosphorus ratio of about 1.2 to 1. There is considerable variation in opinion as to the optimum ratio. Stearns (5) has shown that the calcium-phosphorus ratio varies with nutritive requirements at different ages; i.e., 1.5 : 1 to 2:1 for infants, to about 1:1 for older children. Milk, which provides a sound basis for good nutrition, has a calcium to phosphorus ratio of about 1.2 to 1.

Calcium utilization is favorably influenced by an adequate level of dietary vitamin D and impaired by an excess of magnesium or oxalate in the diet.

Iron and manganese form insoluble phosphate compounds in the intestinal tract and if present in sufficient amounts may result in impaired phosphorus utilization. Similarly, dietary phosphorus in excess can interfere with the utilization of these substances.

McCance, et al (6) has shown that diets high in protein significantly favor the absorption of both calcium and magnesium. This effect of protein may be of practical importance in areas of limited calcium supply. In addition, many proteins of high quality provide an additional amount of dietary phosphorus.

Since a slightly acidic intestinal pH favorably influences the absorption of calcium, lactose (which promotes the establishment of an aciduric bacterial flora) is an important dietary factor in calcium utilization.

The combination of an optimum calcium-phosphorus ratio, a substantial level of high quality protein, and lactose in milk is of no small concern in satisfying the nutritional needs of the rapidly growing child who is laying down bone and muscle at a prodigious rate.

Carbohydrate and Fat and the Balanced Diet

In terms of dietary interrelationships carbohydrate and fat will be discussed with the vitamins. Carbohydrate, the most inexpensive and widely available source of energy provides 50 to 60 percent of the calories of the average diet. Although these figures are subject to wide variations, a reasonable rule of thumb that may be applied is that the carbohydrate intake varies inversely with the economic status and standard of living of a population.

About 93 to 98 per cent of most common fats can be utilized and man can tolerate dietary fat equivalent to at least a third of the average daily energy consumption (7). The level of fat in the diet is today a matter of some concern and has led to an as yet unsettled controversy involving its influence on cholesterol production and the possible etiological significance of this substance to atherosclerosis. Certainly dietary fat is valuable as a concentrated source of calories, for the palatability that it lends to the diet, and as a source of essential unsaturated fatty acids.

Forbes (8) has also shown that on a mixed diet containing fat, animals have extra calories for work, growth, or storage. Fat apparently confers efficiency in utilizing food energy on both growing and mature rats.

Vitamins and Vitamin Interrelationships

As our knowledge of the vitamins becomes greater, it becomes increasingly evident that these substances are important components of enzymes which function in cyclic systems, serving to metabolize carbohydrate, fat and protein for the various physiologic processes. It is easy to appreciate, therefore, that a metabolic process can be most readily interrupted at the point of the weakest link in the cycle or chain of reactions, or at the site of reaction represented by deficiency of a specific vitamin.

It cannot be assumed, however, that the deficiency of one vitamin will necessarily have the same quantitative effect on a metabolic cycle as a deficiency of another, because the reactions in which the respective vitamins are involved may proceed at different rates. Just as the speed of a convoy is limited to that of the slowest ship, so the overall rate of a metabolic cycle is limited to the rate of the slowest reaction in the cycle.

This concept might help to explain the clinical observation that in the case of the B complex vitamins, treatment with the single vitamin indicated by the predominant signs and symptoms in a patient with a deficiency disease may result in the precipitation of severe manifestation of other vitamin deficiencies. In other words, as the most rate-limiting reaction or enzyme level becomes corrected by specific vitamin administration, the effects of the next most limiting reaction can then become manifest. We now recognize

as known or strongly implied components of enzyme systems the following B complex vitamins: niacin, riboflavin, thiamine, pyridoxine, pantothenic acid, folinic acid, vitamin B_{12} and biotin.

Experimental studies, mostly with rats, have shown that the intestinal flora can be influenced by the type of diet fed with respect to carbohydrate and fat and that the vitamin requirements may be altered accordingly. Fredericia (9) first observed that raw—but not cooked—potato starch permitted animals to grow satisfactorily in diets deficient in the B vitamins. A great many similar studies with different carbohydrates permits one to conclude that the vitamin requirement for certain members of the B complex vitamins can be varied considerably depending on the type of carbohydrate used in the diet. In general one might place the carbohydrates in the following decreasing order of effectiveness in stimulating the intestinal synthesis of vitamins and thus reducing the level of vitamin requirements: dextrin, starch, lactose, glucose, and sucrose. This exact order may vary with different vitamins.

The amount of dietary fat may also affect the vitamin requirements. This effect is not necessarily uniform for all vitamins. Evans and Lepkovsky (10) showed that high fat diets reduced the thiamine requirement. Mannering (11) found that increased levels of dietary fat raised the riboflavin requirement of the rat. Gottlieb et al (12) have shown that a high fat diet increases the requirement for vitamin E; and Booth et al (13) showed that certain fatty acids exert a slight antirachitic value, thus reducing the requirement for vitamin D.

It is also of interest to note that fatty acids from marine fats may be injurious to rats and that this effect may be counteracted with riboflavin (14). Marine oils, such as cod liver oil, are suspected of destroying vitamin E in the intestinal tract. Mackenzie et al (15) found that tocopherol fed separately from cod liver oil will prevent the development of muscular dystrophy in rabbits.

It has also been shown that the choline requirement of the animal is increased as the fat content of the diet is increased. This effect is modified, however, if the diet contains an adequate amount of methionine or methionine-containing protein to supply labile methyl groups for the synthesis of choline and for other transmethylation reactions.

Just as vitamins function in close association in enzyme systems in metabolic cycles, their association is also important in the maintenance and development of specific tissues. The development of bone serves to illustrate this point.

Not only is vitamin D important for the development and calcification of bones, but in vitamin D deficiency one sees skeletal lesions which often

involve detachment of the epiphysis from the shafts of bones (16). Mellanby (17) has shown that vitamin A is also concerned in bone formation. A lack of this vitamin results in bones that often become cancellous, overgrown, and deformed. Vitamins A, C, and D are also concerned in tooth development.

Synergism between vitamins must also be considered. It has been shown in several laboratories that vitamin E promotes the efficient utilization of vitamin A. Moore et al (18) first noted that the inclusion of vitamin E in the diet resulted in the increased storage of vitamin A under controlled conditions. Hickman et al (19) also found that mixed tocopherols increased the growth promoting effect of vitamin A alcohol, vitamin A acetate, or cod liver oil. Hickman also found that some antioxidants, among which was ascorbic acid, in addition to the tocopherols shared their ability to cause increased growth in conjuction with vitamin A or carotene.

The above cited relationships between vitamins by no means exhaust the literature, but should serve to indicate the complicated nature of the problem.

Effects of Massive or Unbalanced Dosage of Vitamins

Despite their tremendous physiological activity in sometimes exceedingly small doses, vitamins are "nontoxic" at least in several multiples of even rather large therapeutic doses. An excessive intake of a vitamin results for the most part in its rapid excretion in the urine.

It has been noted above that when an animal or a human being is suffering from multiple dietary deficiencies, treatment with one vitamin may permit development of signs and symptoms of other vitamin deficiencies. Sydenstricker (20) noted that the treatment of pellagrins with niacin alone may lead to the intensification of certain signs of the disease.

This would seem to argue in favor of the rational use of B complex mixtures at dosage levels adequate to meet the needs of each individual case—certainly at therapeutic levels in deficiency diseases or suspected deficiency diseases, or in circumstances of severe stress.

Vitamins A and D are the only vitamins which produce distinctly deleterious effects from unbalanced overdosing, although the levels involved are quite beyond that which could reasonably be expected in routine usage. Over 25 cases of hypervitaminosis A (mostly children) have been reported since this syndrome was first described by Josephs in 1944 (21). Clinically, the syndrome of vitamin A toxicity is characterized by anorexia, loss of weight, irritability, low grade fever, pruriginous rash, sparseness of hair, hepatomegaly and tenderness and swelling over the long bones. There is

no evidence that hypervitaminosis A affects the function of the adrenals, gonads, adipose tissue, endocrine glands or retina. The minimal preclinical latent period of vitamin A poisoning is about 6 months and the minimal toxic daily dose for children is about 75,000 units.

In the reported cases of vitamin A toxicity, a wide dosage range of vitamin A has been used, varying from 60,000 to 600,000 units per day. The time lapse before the onset of toxic symptoms is variable, but is usually a matter of several months. The minimal amount of vitamin A necessary to produce symptoms in the adult is not known, but may be estimated to be in excess of 1,000,000 units as a single dose (21). Recovery from pain and other symptoms is prompt when vitamin A is excluded from the diet.

Even though over 100 cases of hypervitaminosis D have been reported, no exact data for the toxic dose of this substance can be given, due mainly to the fact that the toxic threshold varies considerably among individuals. The toxic range may be stated, however, as from 5,000 to 20,000 units per kilo of body weight per day (22). Severe intoxication has been observed in some patients after 600,000 units. Others did not show toxic symptoms after 5 to 8 million units. Mild disturbances consist of anorexia, nausea and fatigue. Symptoms of severe intoxication are renal damage, hypercalcemia, dehydration and death. Immediate withdrawal of vitamin D on the first indication of toxicity will, as a rule, result in a rapid disappearance of the symptoms. Other treatment which has proved successful includes, in addition to the cessation of vitamin D intake, low-calcium diet, avoidance of alkali and undue exposure to sunlight, and sufficient fluids to insure a daily urinary output of at least 2000 cc.

As to the toxicity of some of the water-soluble vitamins, the following may be cited: Williams and Spies (23) have given as much as 500 mg. of thiamine daily for a month without causing objective symptoms; Kuhn states (24) "Experimental hypervitaminosis B_2 (riboflavin) is not known. Lactoflavin is not poisonous in amounts 1000 times the physiological dose." Rosenberg indicates that an intake of about 1000 times the normal daily intake of niacin may be considered relatively non-toxic. High dosage levels of niacin may produce a flushing of the skin which is not seen with niacinamide; Lowry was able to give 1 gm. of ascorbic acid per day to 4 adults for 3 months. No harmful effects whatever were noted during this period.

While it is true that malnutrition can be caused by imbalances in nutrition resulting from excesses of certain nutrients, the literature on massive and imbalanced overdosage with vitamins makes it exceedingly doubtful that imbalance and harm could result from the reasonable use of vitamins in the fortification of appropriate foods—especially foods that have wide consumer appeal and usage.

Yet another factor which may alter the vitamin requirement and produce dietary imbalance is the presence in diets of antimetabolites. It has been shown (27) that certain chemical substances which have a structural relationship to various vitamins may when administered, cause specific deficiency diseases which can be "reversed by the metabolites in question when given in adequate amounts." That such antimetabolites can exist under certain conditions is demonstrated by the vitamin K deficiency caused by dicoumarol when spoiled sweet clover hay is eaten by cattle. Recently Bean and Hodges (28) have reported success in producing a pantothenic acid deficiency in four volunteer human subjects by giving them omega-methyl pantothenic acid. An appreciation of the essential nature of vitamin B_6 was aided when a deficiency of this vitamin was produced in human subjects by using the antimetabolite desoxypyridoxine (29). A recent symposium on the subject of antimetabolites, their mode of action and therapeutic implications is presented elsewhere (30).

Amino Acid Imbalance; Niacin-Tryptophan Interrelationship

While the term imbalance has been widely used in the field of nutrition its application to amino acids and proteins has been relatively recent. This is rather surprising since some of the earliest work on proteins indicated that the amino acid composition of different proteins varied to a very great degree. For example, proteins such as zein and gelatin were found to be devoid of or very low in certain amino acids such as tryptophan and lysine. These proteins came to be known as incomplete proteins in contrast to those which carried a more favorable ratio of the essential amino acids and were designated as complete proteins. Generally it had been assumed that the lack of one amino acid can be corrected by adding the pure amino acid or proteins which would supply the amino acid in question. There was no general understanding that the level of one amino acid might affect the requirement of another. In 1946 the authors published a paper entitled "The Relation of Amino Imbalance to Niacin and Tryptophan Deficiency in Growing Rats" (31). This paper was based on previous results (32) which demonstrated the growth inhibition resulting from the inclusion of corn in a low protein, niacin deficient ration and the apparent dual role of niacin and tryptophan in counteracting this growth depression. Other work had shown that zein (one of the principal proteins of corn), other proteins such as gelatin, and acid-hydrolyzed proteins free of tryptophan created a syndrome exactly analagous to that produced by corn (33). The growth inhibition produced in all cases was counteracted by the addition of either niacin or tryptophan.

Similar effects have been produced in other species since it has been

shown (34) that the niacin requirement of the chick is influenced by the kind and amount of protein used in the ration. Much work has been conducted in order to establish the exact biochemical relationship between niacin and tryptophan, but it is unnecessary to go into these details in this review. It is sufficient to say that these results indicate that the growth depression caused by corn and non-corn containing rations which contain tryptophan-free proteins have a common basis, namely, an imbalance of amino acids.

Although early work (35) suggested that glycine seemed to be the specific amino acid which was largely responsible for the so-called amino acid imbalance, later work (36, 37) demonstrated that several amino acids were involved. Singal et al (36) reported that the four amino acids, histidine, valine, threonine and lysine produced growth inhibition but reported later (38) that threonine alone gave the effect. Hankes et al, (37) showed that the effect of proteins or protein hydrolysates low in tryptophan could be duplicated by adding 0.078 per cent threonine or 0.208 per cent phenylalanine to the basal ration containing 9 per cent of casein plus 0.2 per cent cystine. Continuing work by these workers (39) showed that the omission of 0.2 per cent L-cystine from the 9 per cent casein ration relieved the growth inhibition due to 0.078 per cent DL-threonine, 2 per cent acid-hydrolyzed casein, 0.208 per cent DL-phenylalanine and 2 per cent glycine. The addition of 0.2 per cent DL-methionine in place of the cystine gave the same effect as the cystine itself. Henderson et al (40) used a diet containing 10 per cent hydrolyzed casein and 0.1 per cent tryptophan and produced a severe growth depression when the threonine was increased from 0.33 to 0.38 per cent. Both niacin and tryptophan were effective in counteracting the inhibition. The growth inhibition obtained by adding the various combinations of amino acids appears to be due to the increased requirement for the limiting amino acids when all others are supplied in adequate or generous amounts. It may also be possible that the presence of certain amino acids may affect the availability of others from the digestive tract. For example, it has been shown that threonine inhibits growth whether injected or fed in the ration while phenylalanine acts only when fed (37). These results make it very easy to explain why diets containing high levels of corn need to be fortified with either niacin or tryptophan rich foods (41, 42), although it is possible to produce conditions when tryptophan must be added to obtain the effect (43).

Amino Acid Imbalance and Liver Damage

Other interesting observations on amino acid balance grew out of the

finding that animals on the low casein diets deficient in niacin and tryptophan showed some accumulation of fat in the liver. This occurred even though the diets contained an adequate amount of choline and added methionine or cystine (44, 45). The addition of either niacin or tryptophan improved the rate of growth but did not have any beneficial effect on the livers. In fact the amount of fat actually increased rather than decreased. The fat content of livers of weanling rats is about 8 to 10 per cent on a dry weight basis. However, after the animals had been on a 9 per cent casein diet containing niacin and tryptophan for two weeks the level of liver fat increased to 25 to 30 per cent. Since gelatin had been used as one protein to increase the tryptophan requirement, naturally gelatin was tried for its activity in preventing the fat accumulation. The addition of gelatin had a very significant effect. When 6 per cent of gelatin was added the level of liver fat was reduced to normal. Since gelatin is known to be a rich source of proline the beneficial effect of proline was tried. Proline was found to be completely ineffective, but as other amino acids were tried it was found that threonine was very effective. A level of .36 per cent of DL-threonine or 0.18 per cent of L-threonine was as effective as 6 per cent of gelatin (46).

Singal and associates (47) used diets containing crystalline amino acids as the sole source of nitrogen and showed that the amount of fat accumulating in the liver varied with the amount of threonine in the diet. When low-protein rations were prepared using proteins other than casein, the beneficial effect of threonine varied indicating that other amino acids are involved (48). The delicate balance of amino acids involved in these studies is evident from the fact that the addition of only 0.1 per cent methionine to a basal ration containing 9 per cent casein without a supplement of S amino acids increased the fat content of the liver from approximately 15 per cent to 25 per cent (49). The effect is especially interesting since methionine has long been recognized as a lipotropic factor. It is obvious that the amino acid balance in these studies is separate and distinct from the effect of choline.

These observations are not limited to weanling rats since a similar accumulation of liver fat is obtained with adult animals when the level of casein is reduced to 5 per cent (50). Again threonine and glycine are effective in preventing the increase in fat content. The practical significance of amino acid imbalance is apparent when the basal diet contains rice as the sole source of protein. Although a marked growth response can be produced in rats fed rice diets when lysine and threonine are added (51) the level which stimulates growth does not prevent the accumulation of liver fat (52). When the level of lysine is increased sufficiently to lower the liver fat to normal, a significant depression in growth results. Later work (53) has shown the retardation in growth produced by the addition of 0.4 per cent L-lysine is prevented

by increasing the levels of leucine, isoleucine, valine and histidine. In an extensive series of studies by Sure on the nutritive value of proteins in different cereals and the effect of amino acid supplementation he (54) has also emphasized the production of amino acid imbalance involving lysine, threonine and valine. Shils et al (55) listed the ability of amino acids to prevent fatty livers in rats subsisting on diets in which the protein was derived from cornmeal, rice, or cassava. They found DL-tryptophan 0.5 per cent, and L-lysine 1 per cent — individually or together — partially or completely prevented the fatty condition. Threonine had little or no effect. They also emphasized that the amino acid requirement for prevention of fatty livers was not correlated with requirements for growth.

Toxicity of Amino Acids Related to Imbalance

Several reports have appeared in the literature (see reference 56) regarding the so-called toxic effects resulting from the addition of an excess of individual amino acids to certain diets for experimental animals. It now appears that some of these effects may be due to amino acid imbalance or antagonism between two amino acids. The first clear-cut evidence in animal nutrition came with observations on the relation between leucine and isoleucine. Using a purified diet containing 9 per cent casein supplemented with 0.1 per cent DL-tryptophan and 0.3 per cent DL-methionine, Harper and coworkers (57) found that the addition of 1.0 per cent L-leucine produced a small growth depression which was completely overcome by the addition of 0.5 per cent DL-isoleucine. The addition of 3.0 per cent L-leucine caused a marked growth depression which was largely, but not completely, overcome by the inclusion of 1.7 per cent DL-isoleucine. Although isoleucine did not completely counteract the effect of 3.0 per cent L-leucine, no growth retardation was observed when this high level of leucine was added to an 18 per cent casein diet (56). The results suggested that the utilization of other amino acids was inhibited by the large excess of leucine or that the toxic effect can be overcome only by increasing the intake of other essential amino acids. The leucine-isoleucine relationship has practical application. Zein, the alcohol-soluble protein of corn, contains approximately 23 per cent leucine. However, analysis of zein indicates an isoleucine content of 7.3 per cent, showing that the ratio is not too wide. In spite of this observation Kligler and Krehl (58) found that zein diets supplemented with amino acids to give levels considered sufficient to meet the amino acid requirement of the rat failed to support a satisfactory rate of growth, and normal growth was obtained only upon addition of casein. Geiger et al (59) also reported that rats fed a zein diet supplemented with all the essential amino acids except leucine and isoleucine

grew at a rate which was still below normal. In 1953, Sauberlich et al (60) found isoleucine to be one of the amino acids most limiting for growth of rats fed corn diets. A recent paper by Benton, Harper and Elvehjem (61) clearly demonstrates that isoleucine is limiting for the growth of rats fed corn diets supplemented with lysine, tryptophan, valine and threonine or 24 per cent zein diets supplemented with most of the essential amino acids. Since 24 per cent of zein in the ration supplies over twice the requirement of the rat for isoleucine it is apparent that isoleucine in zein is only partly available. When diets high in corn are used an amino acid imbalance will undoubtedly be encountered although the effect is due largely to the unavailability of isoleucine, which is needed to counteract the high level of readily available leucine.

Further studies (62) have shown that the more complete effect of casein in counteracting high levels of leucine is due to the valine which is supplied. By using a 9 per cent casein diet and varying the levels of leucine, valine, isoleucine and phenylalanine, evidence was obtained for antagonisms between isoleucine and valine, between phenylalanine and isoleucine and between phenylalanine and valine. These relationships in the rat are very similar to the early observations with bacteria. In 1939 Gladstone (63) working with B anthracis found that an excess of leucine in the medium increased the requirement for valine, that an excess of valine increased the need for leucine and that an excess of isoleucine increased the need for both leucine and valine. Brickson, Henderson, Solhjell and Elvehjem (64) used lactic acid bacteria and demonstrated that high levels of leucine and valine inhibited the utilization of isoleucine for growth and that high levels of leucine and isoleucine reduced the utilization of valine. Recently Dien, Ravel and Shive (65) have demonstrated with bacteria six separate antagonisms among the three amino acids — leucine, isoleucine, and valine.

Benton et al (66) have also shown that the growth of rats fed a 9 per cent casein diet supplemented with tryptophan and methionine is markedly decreased by adding either 3 per cent DL-phenylalanine or 3 per cent of L-tyrosine. A supplement of threonine prevented the growth depression in both cases. Threonine was only partly effective against the more severe growth depression caused by 5 per cent of DL-phenylalanine. Threonine did not prevent the growth depression caused by phenylalanine when tryptophan was not added to the diet.

Mechanism of Amino Acid Imbalance

It is apparent that practically all the amino acids are involoved in some type of imbalance, but the mechanisms of the reactions involved are more

difficult to explain. A meager start has been made to explain some of the effects but much more work is needed. Sauberlich and Salmon (43) studied some of the basic changes that may occur in the tryptophan imbalance condition and found a marked lowering of the level of tryptophan in the blood plasma. The percentage of free and total tryptophan and certain other amino acids excreted in the urine was 2-3 times greater in the deficient animals than in those given tryptophan. This increased loss of amino acids may reflect an attempt by the animal to remove the excess unused amino acids. Beyer et al (67) infused leucine into the blood of a dog and observed that this increased the level of isoleucine excretion in the urine. Kamin and Handler (68) used a similar procedure and found that excretion of isoleucine was increased 140 times when leucine was infused. These effects are not surprising because of the similarity in the structure of leucine and isoleucine. However, the balance between phenylalanine and threonine is more difficult to explain on this basis. Phenylalanine seems to show less specificity and affects the amino acid most limiting for growth. If phenylalanine acts as a specific antimetabolite for threonine a high level should increase liver fat since threonine is needed to maintain normal liver fat. However, it was found that phenylalanine either decreased or did not affect the level of liver fat.

More specific information may be obtained from studies on changes in tissue enzymes after the various nutritional regimens. Williams and coworkers at Wisconsin have undertaken an extensive program on the effect of amino acid deficiencies on enzyme changes in the liver. When several different enzyme activities in the liver can be doubled or halved by adding or omitting 0.36 per cent DL-threonine to the diet (69) it is apparent that slight dietary changes in individual amino acids can influence metabolic processes in a very powerful manner. Other studies have shown that a deficiency of one amino acid may affect liver enzymes in a manner quite different from that produced by a deficiency of another amino acid (70, 71). At present we cannot determine whether the imbalance of amino acids acts directly on the enzyme system or indirectly through the production of a single deficiency.

In conclusion, sufficient experimental evidence has now been accumulated to demonstrate several types of amino acid imbalance. 1. When the most limiting amino acid in a diet poor in protein is increased, a deficiency of the next most limiting amino acid may occur. 2. The addition of a small amount of the second most limiting amino acid may increase the severity of the deficiency of the most limiting amino acid. 3. An excess of one amino acid may reduce the utilization of another amino acid that is provided in normally adequate amounts in the diet to such an extent that a deficiency occurs. 4. An excess of one amino acid may be sufficient to precipitate a

deficiency of other nutrients such as B vitamins. These studies have also emphasized two other points of importance. 1. What may be considered a satisfactory balance of amino acids by one criterion, e.g., growth, may be inadequate when judged by another criterion, e.g., the deposition of liver fat. 2. In contrast to the rather wide ranges tolerated in the case of other nutrients, the toxic levels of many of the amino acids range from 2 to 10 times the requirements. Fortunately from a practical point of view the amino acids supplied by most good quality proteins and most diets used, at least in this country, fall within these ranges.

SUMMARY

The concept of the deficiency diseases opened a new era in nutrition and medicine in the first decade of this century. Developments have been both rapid and extensive with the uncovering of a large group of chemical compounds known as vitamins. Emphasis in the study of nutritional biochemistry has fluctuated among calories, proteins, minerals, vitamins, carbohydrates and amino acids. Each shift of focus brings new knowledge, not only about the subject of investigation, but about the interrelationships of this new fact with the others. Our expanding knowledge makes it clear that indeed biology is a continuum and one cannot tamper with one area without producing shifts and changes in another. Such is the effect of imbalance in nutrition.

REFERENCES CITED

1. National Research Council, "Recommended Dietary Allowances. Reprint and Circular Series No. 129." Washington, D. C. (1948).
2. POLLACK, H. and Halpern, S. L. Therapeutic Nutrition, Prepared with Collaboration of the Committee on Therapeutic Nutrition, Food and Nutrition Board, National Research Council Publication No. 234.
3. WILLIAMS, R. J., Beerstecher, Jr., E., and Berry, L. J. The Concept of Genetotrophic Disease. *Lancet 258,* 287 (1950).
4. ELVEHJEM, C. A., and Krehl, W. A. *J. A. M. A. 135,* 279 (1947).
5. STEARNS, GENEVIEVE. *Am. J., Dis. Child. 42,* 749 (1931).
6. MCCANCE, R. W., Widdowson, E. M., and Lehmann, H. *Biochem. J. 36,* 686 (1942).
7. LONGWORTHY, C. A. *J. Ind. & Eng. Chem. 15,* 276 (1923).
8. FORBES, E. B., Swift, R. W., Elliot, R. F. and James, W. H. *J. Nutrition 31,* 203 (1946).
9. FREDERICIA, L. S., Skandinow. *Arch. f. Physiol. 49,* 129 (1926).

10. Evans, H. M., and Lepkovsky, S. *J. Biol. Chem. 83,* 269 (1929).
11. Mannering, G. J., Orsini, D., and Elvehjem, C. A. *J. Nutrition 28,* 141 (1944).
12. Gottlieb, H. F., Quackenbush, F. W., and Steenbock, H. J. *J. Nutrition 25,* 433 (1943).
13. Booth, R. G., Henry, K. M., and Kon, S. K. *Biochem. J. 36,* 445 (1942).
14. Yoshida, M. *J. Agr. Chem. Soc. Japan, 13,* 120 (1937).
15. Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V. *J. Nutrition 21,* 225 (1941).
16. Hess, A. F. Scurvy Past and Present. J. N. Lippincot Co., Philadelphia and London (1920).
17. Mellanby, E. *J. Physiol. 94,* 380 (1938).
18. Moore, T. *Biochem. J. 34,* 1321 (1940).
19. Hickman, K. C. D., Harris, P. L., and Woodside, M. R. *Nature 150,* 91 (1942).
20. Sydenstricker, U. P. *Ann. Int. Med. 15,* 45 (1941).
21. Josephs, H. W. *Am. J. Dis. Child. 67,* 33 (1944).
22. Reed, C. I. *Am. Pract. and Digest Treat. 1,* 164 (1950).
23. Williams, R. R. and Spies, T. D. Vitamin B_1 In Medicine. N. Y. Macmillan (1938).
24. Kuhn, R. Klin. *Wochschr. 17,* 222 (1938).
25. Rosenberg, H. R. The Vitamins, N. Y. Interscience (1945) p. 249.
26. Lowry, O. H. *Proc. Soc. Exp. Biol. & Med. 80,* 361 (1952).
27. Woolley, D. W. *Science 100,* 579 (1944).
28. Bean, W. B. and Hodges, R. E. *Proc. Soc. Exp. Biol. and Med., 86,* 693 (1954).
29. Mueller, J. F. and Viltez, R. W. *J. Clin. Investig. 29,* 193 (1950).
30. *American Journal of Clinical Nutrition,* Vol. 3, No. 4.
31. Krehl, W. A., Henderson, L. M., de la Heurga, J. and Elvehjem, C. A. *J. Biol. Chem. 166,* 531 (1946).
32. Krehl, W. A., Sarma, P. S., Teply, L. J. and Elvehjem, C. A. *J. Nutrition 31,* 85 (1946).
33. Krehl, W. A., Sarma, P. S., and Elvehjem, C. A. *J. Biol. Chem., 162,* 403 (1946).
34. Briggs, G. M., Groschke, A. C. and Lillie, R. J. *J. Nutrition 32,* 659 (1946).
35. Henderson, L. M., Deodbar, T., Krehl, W. A. and Elvehjem, C. A. *J. Biol. Chem. 170,* 261 (1947).
36. Singal, S. A., Sydenstricker, V. P. and Littlejohn, J. M. *J. Biol. Chem. 171,* 203 (1947).
37. Hankes, L. V., Henderson, L. M., Brickson, W. L. and Elvehjem, C. A. *J. Biol. Chem. 174,* 873 (1948).
38. Singal, S. A., Sydenstricker, V. P., and Littlejohn, J. M. *J. Biol. Chem. 176,* 1063 (1948).
39. Hankes, L. V., Henderson, L. M. and Elvehjem, C. A. *J. Biol. Chem. 180,* 1027 (1949).
40. Henderson, L. M., Koeppe, O. J. and Zimmerman, H. H. *J. Biol. Chem. 201,* 697 (1953).
41. Elvehjem, C. A. *J. Am. Diet. Assn. 24,* 653 (1948).
42. Salmon, W. D. *Arch. Biochem. 51,* 30 (1954).
43. Sauberlich, H. E. and Salmon, W. D. *J. Biol. Chem. 214,* 463 (1955).
44. Singal, S. A., Hazan, S. J., Sydenstricker, V. P., and Littlejohn, J. M. *J. Biol. Chem. 200,* 867 (1953).

45. HARPER, A. E., Monson, W. J., Benton, D. A. and Elvehjem, C. A. *J. Nutrition 50,* 383 (1953).

46. HARPER, A. E., Monson, W. J., Benton, D. A., Winje, M. E. and Elvehjem, C. A. *J. Biol. Chem. 206,* 151 (1954).

47. SINGAL, S. A., Hazan, S. J., Sydenstricker, V. P. and Littlejohn, J. M. *J. Biol. Chem. 200,* 883 (1953).

48. WINJE, M. E., Harper, A. E., Benton, D. A., Boldt, R. E. and Elvehjem, C. A. *J. Nutrition 54,* 155 (1954).

49. HARPER, A. E., Benton, D. A., Winje, M. E. and Elvehjem, C. A. *J. Biol. Chem. 209,* 159 (1954).

50. HARPER, A. E., Benton, D. A., Winje, M. E., Monson, W. J. and Elvehjem, C. A. *J. Biol. Chem. 209,* 165 (1954).

51. PECORA, L. J. and Hundley, J. M. *J. Nutrition 44,* 101 (1951).

52. HARPER, A. E., Winje, M. E., Benton, D. A. and Elvehjem, C. A. *J. Nutrition 56,* 187 (1955).

53. DESHPANDE, P. D., Harper, A. E., Quiro-Perez, F. and Elvehjem, C. A. *J. Nutrition* (In press).

54. SURE, B. *J. Ag. and Food Chem. 2,* 1108 (1954).

55. SHILS, M. E. and Stewart, W. B. *Proc. Exp. Biol. and Med. 87,* 629 (1954).

56. HARPER, A. E., Benton, D. A. and Elvehjem, C. A. *Arch. Biochem. and Biophys. 57,* 1 (1955).

57. HARPER, A. E., Benton, D. A., Winje, M. E. and Elvehjem, C. A. *Arch. Biochem. and Biophys. 51,* 523 (1954).

58. KLIGLER, D. and Krehl, W. A. *J. Nutrition 41,* 215 (1950).

59. GEIGER, E., Courtney, G. W., and Geiger, L. E. *Arch. Biochem. and Biophys. 41,* 74 (1952).

60. SAUBERLICH, H. E., Chang, W. and Salmon, W. D. *J. Nutrition 51,* 623 (1953).

61. BENTON, D. A., Harper, A. E. and Elvehjem, C. A. *Arch. Biochem. and Biophys. 57,* 13 (1955).

62. BENTON, D. A., Harper, A. E.,Spivey, H. E. and Elvehjem, C. A. *Arch. Biochem. and Biophys.* (In press).

63. GLADSTONE, G. P. *Brit. J. Exp. Path. 20,* 189 (1939).

64. BRICKSON, W. L., Henderson, L. M., Solhjell, I. and Elvehjem, C. A. *J. Biol. Chem. 176,* 517 (1948).

65. DIEN, L. T. H., Ravel, J. M. and Shive, W. *Arch. Biochem. and Biophys. 49,* 283 (1954).

66. BENTON, D. A., Harper, A. E., Spivey, H. E. and Elvehjem, C. A. *Arch. Biochem. and Biophys.* (In press).

67. BEYER, K. H., Wright, L. D., Skeggs, H. R., Russo, H. F. and Shaner, G. A. *Am. J. Physiol. 151,* 202 (1947).

68. KAMIN, H. and Handler, P. *Am. J. Physiol. 164,* 654 (1951), *169,* 305 (1952).

69. HARPER, A. E., Monson, W. J., Litwack, G., Benton, D. A., Williams, J. N. Jr. and Elvehjem, C. A. *Proc. Soc. Expt. Biol. and Med. 84,* 414 (1953). Arata, D., Harper, A. E., Svenneby, G., Williams, J. N. Jr., Elvehjem, C. A. *Proc. Soc. Expt. Biol. and Med. 87,* 544 (1954).

70. BOTHWELL, J. W. and Williams, J. N. Jr. *J. Biol. Chem. 191,* 129 (1951).

71. PRIGMORE, J. R., Bothwell, J. W. and Williams, J. N. Jr. *Proc. Soc. Expt. Biol. Med. 88,* 43 (1955).